

Structures of Enzymes in the Alginate Biosynthetic Pathway of *Pseudomonas aeruginosa*.

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Abstract No. snoo1133

Beamline(s): X8C, X12B

Introduction: Cystic Fibrosis (CF) patients are prone to secondary infections in the lungs. One of the infectious organisms is *Pseudomonas aeruginosa*. In the CF lung this organism transforms into a mucoidy state that overproduces its natural alginate coating - a polysaccharide. In this state *P. aeruginosa* is resistant to antibiotics and the innate immune response of the lung. Various proteins are involved in the synthesis of alginate (Shanker *et al.* 1995). These proteins have not been structurally characterized and the understanding of their mechanisms would be greatly enhanced by the structural knowledge obtained.

Methods and Materials: In this study we have obtained large single crystals of two native recombinant proteins involved in the alginate pathway for X-ray diffraction studies. These are the Phosphomannomutase - PMM (Regni, 2000) and GDP-mannose Dehydrogenase -GMD proteins. Seleno-methionine substituted protein crystals or heavy atom soaked crystals will be used for multiple-wavelength anomalous dispersion (MAD) phasing experiments. Protein complexes, with substrates and cofactors, have been obtained by crystal soaking experiments. All data collection has been carried out at 100-110 K.

Results: We have obtained a 1.75Å native data set and a 2.1Å Se-MAD data set of PMM. The phases for this structure have now been solved and model-building/refinement is proceeding. Data sets of PMM complexed with its various cofactors and substrates have been obtained to near completeness with maximal resolutions of 1.8 to 2.1Å. The GMD crystal form that diffracts to high resolution has a long unit cell axis (>300Å). Data collected for GMD includes a 2.6Å mercury substituted data set that is non-isomorphous with a high-resolution data set collected at APS. We confirmed the incorporation of selenium in GMD.

Conclusions: We have been able to progress rapidly with the structure solution of PMM and are beginning to investigate the complex data sets. The GMD is progressing but is limited due to the need for detectors with a 2-theta swing to collect high-resolution data.

Acknowledgments: Proteins were provided by Dr. P.A. Tipton. Work supported by the University of Missouri and the National Institutes of Health.

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